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Chapter 7

THE APPLICATION OF DNA MICROARRAYS IN THE FUNCTIONAL GENOMIC STUDY OF SCHISTOSOME/HOST BIOLOGY

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Key words:

1. INTRODUCTION

The end of the 20th century and the start of the new millennium have bore witness to a remarkable revolution in the way parasite/host biological interactions can be conceptually designed and experimentally studied. Although most traditional investigations of parasitism have been motivated by hypothesis- or model-driven science, the recent successes of parasite and host expressed sequence tag (EST)/genomic sequencing projects have opened up a new avenue to the parasitologist – discovery driven science. Even the most steadfast, classically-trained parasitologist can see enormous value in coupling conventional experimental techniques with the new and exciting technologies made possible by genome sequencing efforts. In this era of functional genomics (defined as experimental approaches that use genomic structural information to understand biology in a systemic and comprehensive fashion (1)) the genome-wide analysis of mRNA expression using DNA microarrays has become pivotal. Here, we review the impact DNA microarrays have had on recent schistosome/host investigations and outline the exciting future areas we plan to visit in our study of schistosome sexual maturation, developmental biology, gender interactions, and host immuno-biology.

2. DNA MICROARRAYS

Use of glass slide DNA microarrays to study transcriptional changes between two different nucleic acid samples obtained from eukaryotes was first reported in 1995-1996, when Patrick Brown's group at Stanford University published a series of seminal observations on *Arabidopsis* and *Saccharomyces* gene expression (2, 3). These high profile investigations brought DNA microarray technology worldwide publicity, building on the principles first conceived by Ekins and Chu during their development of solid-support immunoassays in the 1980's (4). DNA microarray technology has exploded since these early reports of its usage and all aspects of this evolving functional genomics tool have recently been critically re-reviewed in *Nature Genetics* (Supplement Volume 32, 2002).

A major advantage of utilizing DNA microarrays to study gene expression in the functional genomics era is that current technological improvements in robotics have made it possible to systematically array thousands of DNA elements onto chemically defined matrixes. In some cases, whole genomes of pathogenic organisms can be represented on a single glass slide (5). This means that gene expression profiles of whole genomes can be assayed simultaneously, exponentially increasing the amount of biological information obtained every time a single experiment is performed. However, highly informative DNA microarray studies have already been performed on partial parasite genomes (eg. *Plasmodium* (6)); this will continue to be the trend especially for those parasite species where complete genome annotation is unlikely in the near future (eg. *Ascaris*, *Echinococcus*, and *Trichuris*).

The vast amounts of sequence information available for mouse and man has made DNA microarrays from these schistosome definitive hosts an additional attractive resource for numerous questions related to parasite/host interactions. These questions might include how immunopathology develops, how immune responses are induced and regulated during infection, and what immune correlates are associated with resistance and vaccine induced protection. Together, the combined use of schistosome and host DNA microarrays in modern parasitological experimentation will be instrumental in generating comprehensive views of the mechanisms influencing host/parasite immuno-biology.

Two of our recent studies utilizing DNA microarrays have provided insight into the immunopathology of murine schistosomiasis (7) as well as the sexual dichotomy of adult parasites (8). As these two studies provided the framework for our continued interest in using DNA microarrays for future investigations into schistosome biology, they will be discussed below with attention given to the major findings included in each investigation.

3. STUDYING EGG-INDUCED IMMUNO-PATHOLOGY IN MURINE SCHISTOSOMIASIS USING cDNA MICROARRAYS

The schistosome egg is the causative agent that precipitates most severe forms of immuno-pathology in the murine model of schistosomiasis (see chapters 8 and 9). Distinct forms of clinical disease exist in the mouse model and these differences are attributable to the specific type of immune reactions developing in response to schistosome eggs (recently reviewed (9)). For example, schistosome-infected interleukin 10 (IL-10)/IL-12 (type-2 polarized) and IL-10/IL-4 (type-1 polarized) – deficient mice display distinct, and non-overlapping egg-induced pathologies, yet both groups of animals suffer from elevated mortality rates (10) in comparison to infected WT (wild-type) mice. Type-1 polarized animals display 100% mortality at 8 weeks post-infection and exhibit elevated splenic iNOS activity, increased production of pro-inflammatory cytokines (found in both liver and serum), decreased hepatic fibrosis, increased levels in circulating liver transaminase, and rapid cachexia. In contrast, the type-2 polarized animals develop a progressive wasting disease that correlates with increased production of the pro-fibrotic cytokines IL-4 and IL-13, augmented hepatic fibrosis, and significant mortality (~ 50%) during the chronic stages of infection. For greater insight into the genetic differences associating with these diverse egg-induced pathologies, a murine cDNA microarray fabricated from 2200 unique DNA elements was used to obtain gene expression profiles from hepatic tissue harvested from both infected mouse strains (7). Profiles obtained for the infected type-1 polarized mice illustrate that aberrant regulation of the apoptotic machinery, increased recruitment of activated macrophages and neutrophils, and diminished ability to transcribe genes associated with collagen production are likely contributing to the egg-induced hepatic pathology. In contrast, hepatic transcriptional analysis of the infected type-2 polarized animals clearly demonstrate that genes involved in wound repair, extra-cellular matrix remodeling, and collagen deposition are induced to a much greater degree than observed for the type-1 polarized mice. These findings thus provided a further mechanistic explanation for the increased mortality rates in type-2 polarized mice and illustrate that type-2 responses promote and type-1 responses inhibit gene transcriptional pathways associated with liver collagen synthesis and matrix remodeling. Furthermore, this study produced a list of candidate genes involved in egg-induced pathology and details how gene expression profiles can lead to insight (role of apoptosis, activated neutrophils and macrophages) into previously unappreciated disease mechanisms that contribute to pathogenesis during infection with schistosomes.

4. STUDYING SCHISTOSOME SEXUAL MATURATION USING cDNA MICROARRAYS

Shortly after the above immuno-pathological study, we became interested in using DNA microarrays to examine schistosome sexual maturation and developmental biology. The two experimental disciplines are logically linked, as murine pathology can be directly related to the biology of parasite sexual maturation. Specifically, adult male schistosomes induce sexual maturation in females (11), which leads to the production of the 100-300 eggs/worm pair every day during infection with this trematode. Any additional information relating to the processes regulating or associating with schistosome sexual maturation and development can be instrumental in understanding, and eventually controlling the immuno-pathology of disease. In a preliminary experiment aimed at identifying novel sex-associated gene transcripts, we fabricated a small *S. mansoni* cDNA microarray from three cDNA libraries originating from two different parasite developmental stages and probed these DNA microarrays with sexually mature male and female material (8). Although the fabricated cDNA microarray consisted of less than 10% of the predicted ORF content of the *S. mansoni* genome, this study expanded the list of gender-associated gene transcripts from all previous studies by a factor of two and provided novel research avenues towards our understanding of the molecules involved in egg-production, tegumental biology, and other sex-related biological processes. We, and others, are in the process of functionally characterizing the biological importance of many of these differentially expressed transcripts.

Due to this pilot experiment's success, we have recently begun the fabrication of an expanded *S. mansoni* oligonucleotide DNA microarray (Fig 1, panel I - containing ~ 50% of the predicted ORF genome content) as well as a small *S. japonicum* cDNA microarray (Fig 1, panel V - 456 unique cDNA clones or ~ 3% of the predicted ORF genome content). These two resources will be extensively used over the next several years to aid our functional genomic research of schistosome biology (discussed in greater detail below).

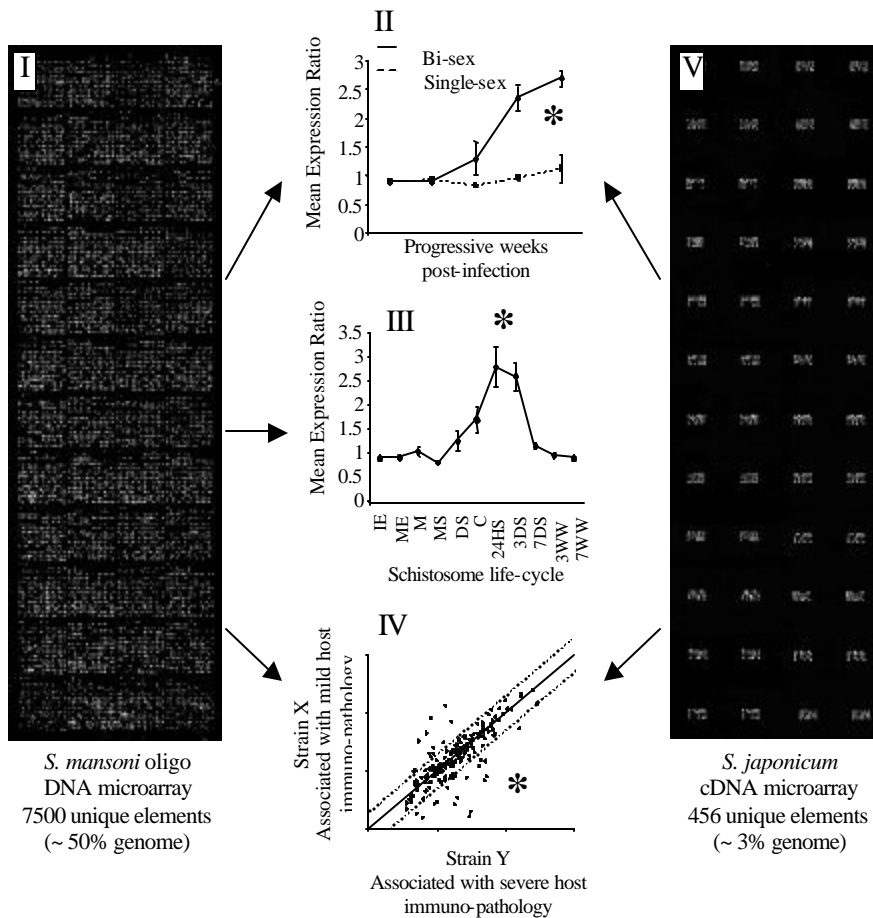


Figure 7-1. Schistosome specific DNA microarrays that will be used in future functional genomic studies of host-schistosome interactions. (*S. mansoni* oligonucleotide DNA microarrays containing 7500 unique elements (I) and *S. japonicum* cDNA microarrays containing 456 unique elements (V)) will be used to identify transcripts associated with: (II) sexual maturation, (III) developmental expression, and (IV) immuno-pathology. (II) An example of one planned sexual maturation study result where * indicates average gene expression profile of transcripts only induced in sexually mature female schistosomes obtained from bi-sex infection experiments. (III) An example of one planned developmental expression profiling result where * indicates average gene expression of transcripts highly induced in 3 day through 7 day schistosomula. IE – immature egg, ME – mature egg, M – miracidia, MS – mother sporocyst, DS, daughter sporocyst, C – cercaria, 24HS – 24 hr schistosomula, 3DS – 3 day schistosomula, 7DS – 7 day schistosomula, 3WW – 3 week sexually immature worms, 7WW – 7 week sexually mature worms. (IV) Scatterplot of gene expression ratios obtained from 2 schistosome strains capable of inducing diverse immunopathology; * indicates those genes associated with the more virulent strain of parasite.

5. WHAT'S NEXT AND EXCITING FUTURE PROSPECTS

Based on the above-described studies, we are excited about this technology's impact on future parasitological investigations. We plan on using parasite DNA microarrays to 1) continue studies into the sexual biology and developmental maturation of schistosomes, 2) investigate whether parasite gene expression profiles can be predictive of virulence and/or immuno-pathology, and 3) identify potential new immuno-prophylactic or chemotherapeutic targets. In addition to these investigations using parasite DNA microarrays, we also are currently using both human and murine DNA microarrays to study immune response induction and regulation during praziquantel chemotherapy of infected hosts. Transcriptional results obtained from these studies, in combination with co-acquired immunological and parasitological data, will shed light into the mechanisms associating with host resistance, susceptibility, and the generation of a dominant and controlled type-2 response. The interpretation of data collected from both parasite and host DNA microarray studies will be instrumental in developing new hypotheses that aid in the characterization of complex, infection-associated, biological interactions.

6. FUTURE USE OF SCHISTOSOME DNA MICROARRAYS

The goals of our future sex-associated studies are to use the above-described schistosome DNA microarrays to identify: 1) genes transcribed in sexually mature adult male and female parasites, 2) genes longitudinally transcribed during sexual development and maturation, and 3) genes differentially regulated in murine infections initiated by single sex versus dual-sex parasite inoculums. In addition to expanding the list of transcripts that associate with adult *S. mansoni* males and females, we plan on identifying adult gender-associated gene products from *S. japonicum* worms, where even less is known about its sexual maturation and egg biology. We therefore hope to contribute to the identification of sex-associated gene transcripts in both species of schistosome.

Although bimodal (adult male versus female) experiments will lead to the cataloguing of many additional adult gender-associated transcripts, we are equally interested in identifying those genes that are longitudinally regulated from immature through mature forms of these dioecious parasites. The timing of gene expression will provide important clues into the development

of sexual maturity, conjugal biology, oviposition, and host/parasite interactions (12). Gene transcripts that demonstrate female associations in the bimodal experiment described above can be additionally segregated into those that are temporally expressed before and after egg development. Therefore, male and female gene expression profiles will be obtained from worms harvested from infected murine hosts at progressive weeks post-infection and probed across the DNA microarrays. These experiments will help elucidate the transcriptional events associated with the acquisition of schistosome sexual maturity.

Although it is envisioned that sex-associated transcription kinetics will be characterized in the above experiments, a third biological area to be addressed during our studies of schistosome sexual biology will involve identifying those genes differentially regulated in single-sex (hosts only infected with one parasite gender) versus dual-sex (hosts infected with both parasite genders) murine infections (*, Fig. 1, panel II). It has been shown that female parasites depend upon direct interaction with male schistosomes to mature into a highly proficient egg-laying organism (13). Conversely, the male schistosome seems capable of normal sexual development in the absence of the female, but some studies suggest that antigenic and biochemical changes do occur (14, 15). In both cases, the genetic machinery affected by maturing in the absence of the opposite sex has yet to be thoroughly dissected. To address this important subject, female and male parasites from single sex infections of each (16) will be harvested from murine hosts at progressive weeks post-infection. The longitudinal pattern of gene expression will be assessed from each separate sex pool of RNA and then compared to those pools of separate sex RNA harvested from a dual sex murine infection via a common reference. It is anticipated that these experiments will uncover contributions of previously unappreciated mechanisms involved in schistosome sexual maturation pathways, which depend upon gender interactions.

To complement these studies of schistosome sexual biology, we plan to use the *S. mansoni* DNA microarrays to profile gene expression across the parasite's lifecycle and identify gene products associated with different parasite strains. The goal of lifecycle expression profiling is to identify coordinated clusters of gene transcripts that associate with each particular life-stage. Using this approach, we will be able to characterize the developmental biology of this pathogen to a large degree, which ultimately will identify transcripts involved in housekeeping functions, uniquely utilized metabolic pathways, infection of intermediate and definitive hosts, and modulation of protective immune mechanisms (Fig. 1, panel III). Further detailed elucidation of these genes' functions may provide insight into novel strategies for immunological or drug intervention.

Finally, we plan to use both *S. mansoni* and *S. japonicum* DNA microarrays as tools to profile gene transcription differences and similarities among various collected field isolates (strains) and laboratory adapted parasite strains. Information obtained from these investigations, in association with other field- and laboratory- collected parameters, may provide significant information related to the virulence of the profiled parasite strains useful in interpreting complicated immuno-epidemiological associations (*, Fig. 1, panel IV). Taken together, data accumulated from our future use of schistosome DNA microarrays will provide novel, testable hypotheses related to diverse biological processes concerning host-parasite interactions, developmental biology, virulence, and sexual maturation of these pathogenic organisms.

7. FUTURE USE OF HUMAN AND MOUSE DNA MICROARRAYS

Investigations using host specific DNA microarrays are also currently being used in our laboratory to study immune response regulation during chemotherapeutic treatment. Because any vaccine administered to a human population will likely be preceded by chemotherapeutic clearance of an active infection, understanding the effect of drug therapy on the host's immune system is an important issue to investigate and will ultimately contribute to the development of an effective immunoprophylactic treatment. The present drug of choice for treatment of all human schistosomes is praziquantel. While the exact mode of action of praziquantel is still not clear, experimental mouse studies (17-19) supported by human investigations (20), suggest that praziquantel therapy induces a protective immune response. However, according to human re-infection studies, this protective immune response may not be long-lived in many individuals (eg. children have increased risk of becoming re-infected) (21, 22). Consequently, characterization of the immunological mechanisms induced by praziquantel treatment that contribute to worm elimination should be more carefully and thoroughly examined. A study of this type would not only provide information critical to schistosome vaccinologists, but would also furnish the immunologist with insights into how the immune system regulates a potentially deleterious response against thousands of parasite antigens that are simultaneously released into the blood at the time of chemotherapy.

Studies to date dissecting the human immune responses longitudinally after praziquantel treatment have been limited. Most have examined the effect of chemotherapy on some aspect of the host immune system (using

sera, PBMCs, or whole blood assays) at periods of several weeks (20, 23), months (22, 24, 25), or years (21, 25) after treatment. Although the majority of these studies have revealed the up-regulation of IgE and IgG responses as well as increased eosinophilia after praziquantel treatment, all of them have neglected to examine the early events induced immediately following chemotherapy. Speculatively, these early host immuno-biological responses to dying worms could directly influence the magnitude of later events including the development of heightened antibody titers, proliferation of eosinophils, and ultimately, generation of immunity to a subsequent infection.

Our current studies involve examining the host's transcriptional profile longitudinally during praziquantel treatment, focusing on immediate as well as late time-points (Fig. 2). In the murine studies, where careful control of the experimental procedure can be achieved, we are currently examining splenic, bonemarrow, and mesenteric lymph node expression profiles during praziquantel treatment in both infected as well as uninfected animals. A DNA microarray consisting of 7445 unique elements (~33% of the predicted ORF content of the genome) will be utilized for these studies in the mouse (Fig. 2 panel III). Our human studies focus on a well-characterized cohort of individuals living on Lake Albert in Uganda, Africa. Adults living in this area are all infected with *S. mansoni* and have previously been shown to homogeneously mount a rapid and vigorous immune response against parasite antigens following chemotherapy. Because of a high infection rate and rapid post-treatment immune response, these individuals provide a unique opportunity to thoroughly characterize the extent, quality, and magnitude of gene activity induced or repressed by chemotherapy and dissect how these differentially regulated genes influence the development of later immunological events (*, Fig. 2, panel II). Here, we are using a DNA microarray (Fig. 2, panel I) consisting of 10560 unique elements (~33% of the predicted ORF content of the genome) and are specifically focusing on gene expression profiles obtained from whole blood (where the red blood cell component has been effectively removed). The contribution of information collected from both murine and human experiments via DNA microarray analysis of selected tissues, in combination with classical immunological and parasitological studies, will help build a more robust picture of the immunological cascade triggered by praziquantel treatment of infected and uninfected individuals. This information should prove useful towards our advanced understanding of praziquantel's protective mechanism of action as well as provide important clues to the gene products that may be associated with resistance to re-infection; both topics remain a priority among planned future immunological investigations.

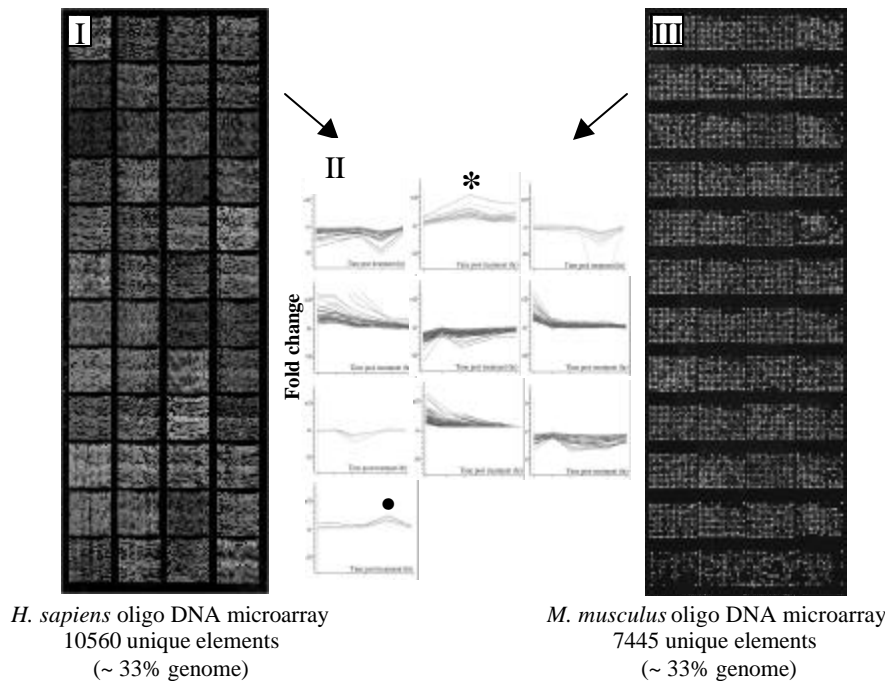


Figure 7-2. Host specific DNA microarrays (*H. sapiens* oligonucleotide DNA microarrays containing 10560 unique DNA elements (I) and *M. musculus* oligonucleotide DNA microarrays containing 7445 unique DNA elements (III)) will be used as a tool to identify patterns of gene expression differentially regulated upon praziquantel clearance of an active schistosome infection. (II) K-means supervised clustering method used to segregate gene expression profiles differentially regulated during praziquantel treatment of schistosome-infected hosts. * Indicates genes induced early after praziquantel treatment and ? indicates genes induced later after drug therapy (possible effector or regulatory transcripts). Other patterns of gene expression influenced by drug therapy can be visualized in the additional clusters.

8. CONCLUDING REMARKS

Although gene expression results obtained through the use of DNA microarrays will undoubtedly increase the path and scope of many future parasite/host investigations, there is still an obvious need to maintain the use of traditional experimental techniques in this functional genomics age. How else would the gene products identified by DNA microarray investigations be further studied in the laboratory setting if we do not plan on using this wealth of information for testing hypotheses by current immunological,

biochemical, and molecular biological techniques? In addition to classical techniques, new methodologies can be used to manipulate parasite and host gene expression. Transgenic or gene deficient cell lines or animals are well established and RNAi and transient/stable transfection of parasites and host cells are being developed which collectively allow for the functional importance of selected gene products to be determined directly.

Each investigator considering the use of DNA microarrays will additionally face the rewarding task of sifting through huge amounts of gene expression information to tease apart biologically significant relationships. The evolution of new bioinformatic approaches for DNA microarray data analysis will dramatically increase the speed in which these biological relationships are identified as well as provide novel opportunities for developing collaborative relationships between the biologist and the mathematician. In terms of future research on schistosomiasis, DNA microarrays and other functional genomic tools have the profound potential to positively impact all areas, provided widespread use is routinely adopted alongside our traditional immuno-parasitological methodologies.

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